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Epidemiology of tattoo skin disease in bottlenose dolphins *Tursiops truncatus* from the Sado estuary, Portugal

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ABSTRACT: We report on the epidemiology of tattoo disease in a community of bottlenose dolphins *Tursiops truncatus* from the Sado estuary, Portugal. The presence of tattoos (T++) and tattoo-like (T+) lesions was examined in 586 photographic records of 35 dolphins taken from 1994 to 1997. Images were rated into 3 categories: good (GI), average (AI) and poor (PI). Dolphins positive for T++ lesions were observed in 19 GI. Dolphins with T+ lesions were seen in 39 GI, 23 AI and 6 PI. For statistical analysis the dolphins were divided into 2 age classes (immature and adult) and the data grouped into 2 periods (1994–1995 and 1996–1997). Minimum prevalence of T++ lesions in 32 dolphins was 21.9% in 1994–1995 and 15.6% in 1996–1997. Variation in prevalence of tattoo disease between the 2 age classes was examined for each period, excluding animals with T+ lesions or considering them either positive or negative for tattoos. Prevalence of the disease was significantly higher in immature dolphins than in adults during both periods, except in the first one when T+ lesions were considered as true tattoos. Temporal variation in prevalence of tattoo disease was examined in 23 adults. Prevalence was significantly higher in 1994–1995 (39.1%) than in 1996–1997 (17.4%). Differences in the number and quality of pictures did not cause significant biases that could have favoured the detection of lesions between age classes or periods. Minimal persistence of the disease ranged between 3 and 45.5 mo. The lesions converted into light grey marks when healing, but may recur. The presence of very large lesions in 2 adult dolphins affected for years may be related to the contamination of the estuary. The high prevalence of the disease, its long persistence, as well as higher frequency in immature individuals, suggest that it is endemic in bottlenose dolphins from the Sado estuary. The contribution of tattoo disease to the decline of this community should be investigated. Three of the 5 dolphins that died during this study had T++ and T+ lesions.

KEY WORDS: Tattoos · Poxvirus · *Tursiops truncatus* · Epidemiology · Disease · Skin · Photo-identification · Portugal

INTRODUCTION

Tattoo disease is characterised by very typical, irregular, slightly in relief, grey, black or yellowish skin lesions known as 'tattoos', which may occur on any part of the body in toothed whales. It has been observed in several species of free-ranging odontocetes from the North Atlantic, East Pacific and Mediterranean Sea, as well as in captive bottlenose dolphins *Tursiops truncatus* (for a review see Van Bressem et al. 1999). In the bottlenose dolphin, Atlantic white-sided dolphin *Lagenorhynchus acutus*, dusky

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dolphin *Lagenorhynchus obscurus*, long-beaked common dolphin *Delphinus capensis*, killer whale *Orcinus orca* and Burmeister's porpoise *Phocoena spinipinnis* the disease is caused by uncharacterised poxviruses (family *Poxviridae*) that are possibly antigenically related to cowpox virus, a member of the *Orthopoxvirus* genus (Geraci et al. 1979, Van Bressem et al. 1993, 1998, 1999, Van Bressem & Van Waerebeek 1996). It is likely endemic (see Thrusfield 1986) in the long-beaked common dolphin, dusky dolphin, offshore bottlenose dolphin and Burmeister's porpoise from Peruvian waters, and may be equivalent to a 'children's disease' in the 3 Delphinidae species from this ocean province. In the dusky dolphins and Burmeister's porpoises, calves may be protected from the disease by maternal immunity (Van Bressem & Van Waerebeek 1996).

Over the last 20 yr, a small community of long-term resident bottlenose dolphins inhabiting the Sado estuary region in Portugal has been studied by photo-identification (dos Santos & Lacerda 1987, Gaspar 1994, 2000, Harzen 1995, 1998, Gaspar & Hammond 2001). The size of this community has decreased from 40 animals in 1986 to an average of 32 in the late 1990s (Gaspar 2000, Gaspar & Hammond 2001, R. Gaspar et al. unpubl. data). Recently, a high prevalence of skin disorders was reported among these animals (Gaspar 1995, Harzen & Brunnick 1997, Wilson et al. 1999a) and typical tattoo lesions were identified in some of them (Gaspar & Van Bressem 1998, Van Bressem & Gaspar 1999). Here we describe the epidemiology of tattoo disease in 35 dolphins from this community using serial photographic records (see Thompson & Hammond 1992) taken in 1994–1997 and discuss the potential impact of the infection on these animals.

MATERIAL AND METHODS

Collection and processing of images. A total of 586 images from 35 dolphins taken during 94 boat surveys in the Sado estuary region (38° 29′ N, 8° 55′ W) between February 1994 and December 1997 were carefully examined for the presence of tattoos (T++) and tattoo-like (T+) lesions. The slides and pictures were taken with motor-driven 35 mm auto- and manual-focus Nikon cameras, and 100–400 mm zoom lens. ISO 100 or 200 Kodak and Fuji colour slide and picture films were used throughout. For convenience, most slides were converted to pictures or digitalised using a FLEXTIGHT Scanner Precision II. In 1994–1995, 95.4 % (N = 153) of the images examined were printed pictures or slides, while 93.4 % (N = 423) of those considered in 1996–1997 were digitalised pictures. Slides of skin lesions taken at necropsy were also available for one dolphin (SIC) that died in 1996. Printed pictures were examined with the naked eye and with use of a magnifying glass (3×). A slide projector and a Corel PHOTO-PAINT (Version 7) program were used to examine the slides and digitalised pictures, respectively. All images were examined for photographic quality. They were rated for usefulness and quality according to a combination of closeness and sharpness into 3 categories: good (GI), average (AI) and poor (PI) images.

Dolphins. All dolphins were individually identified from natural marks present on the dorsal fin and body (see Würsig & Jefferson 1990). Newborn calves were also identified by the close presence of their mothers. Large, robust dolphins with a dark grey colouration were considered as adults (Wilson et al. 1999b; Table 1). Dolphins of similar or somewhat smaller body length, but with a less massive body form and paler skin, were considered as juveniles (APA, ORL, SIC, SUP) (Wilson et al. 1999b). Small dolphins that had a substantially paler colouration than the adults (Wilson et al. 1999b) and that showed a close association with an adult for several years were regarded as calves (BOL, ECL, ESC, ESP, ZOE) (Scott et al. 1990, Wells & Scott 1990, Smolker et al. 1992). One dolphin (EAG) was a calf in 1994 but left its mother in February 1995 (Gaspar unpubl. data) and was considered as a juvenile thereafter. During the study period 2 juveniles (ORL, SIC) and 3 adults died (body found: CAR, LIS) or disappeared (likely dead but body not found: FUG) and 4 calves (BOL, ECL, ESC, ESP) were born.

Both sides of the dolphins were photographed. The body areas most commonly photographed during this study were the back (98.6 % of total photographic records [N = 586] from live dolphins), dorsal fin (94.5 %) and flanks (73.9 %), followed by the head (21.0 %) and tailstock (14.0 %). The belly (1.4 %), tail (1.0 %) and flippers (0.3 %) could only be examined occasionally. In 1994–1995, pictures of both sides of the back, dorsal fin and flanks were available for 75 % (N = 32) of the dolphins, while pictures of at least one side of these body parts were usable for the remaining 25 %. Pictures of both sides of the back, dorsal fin and flanks were available for all the dolphins examined in 1996–1997.

Tattoos and tattoo-like lesions. T++ lesions were identified on the basis of their typical appearance, i.e. irregular, dark grey, black or yellowish marks with a stippled pattern (Fig. 1a). Other marks that looked very similar, but for which some of the previous distinctive characteristics could not be discerned, were qualified as T+ (Fig. 2a). A dolphin was considered positive for tattoo skin disease when at least 1 T++ lesion was detected on its body, likely positive when at least 1 T+ lesion was observed, and negative when no T++ or T+

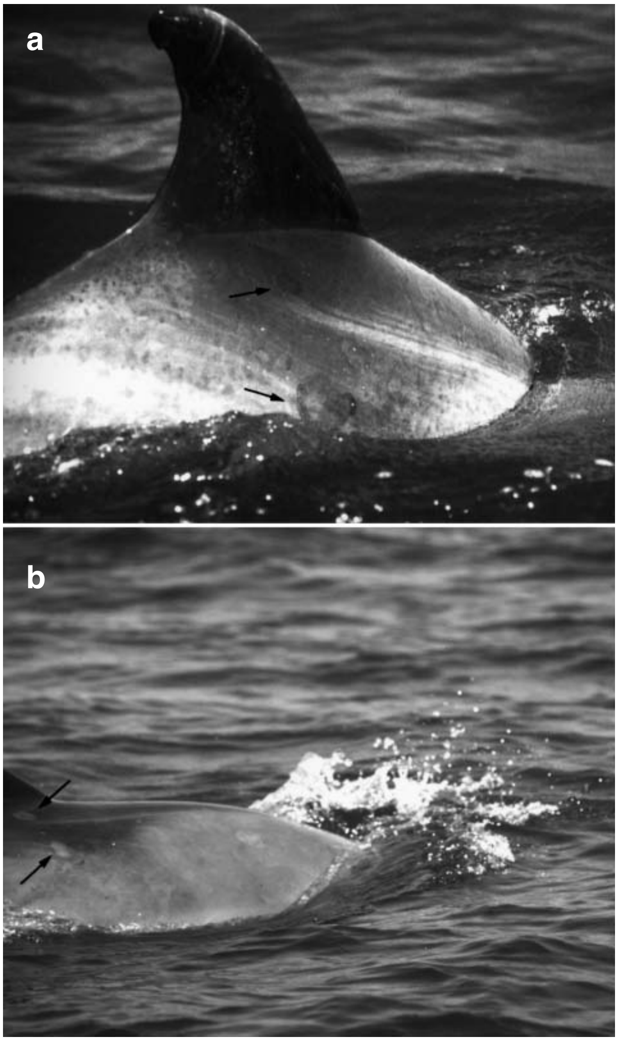

Fig. 1. *Tursiops truncatus.* (a) Tattoo lesions on the back and flank (arrows) of Calf APA in 1996 and (b) remains of the same lesions (G-marks; arrows) in July 1997

lesions could be seen in pictures of several parts of its body. However, because the entire body of the animals could not be examined, all negative results should be regarded with caution. The evolution of individual T++ and T+ lesions was followed through the 4 yr period. In all dolphins positive for these lesions, the body parts affected in 1994–1995 were also photographed in 1996–1997. When all T++ or T+ lesions had disappeared or evolved into different marks, and when no new T++ or T+ lesions were observed in several pictures, a positive dolphin was considered to have cleared the disease. The minimum persistence time (MPT) of a T++ or T+ lesion, i.e. the time during which a particular lesion could be observed repeatedly during the study period, was examined. The MPT of the disease, i.e. the time during which at least 1 T++ or T+ lesion could be detected on a dolphin during the study period, was determined. The topography of T++ and T+ lesions as well as their size (small, medium, large and very large) relative to the body of the dolphins were registered. The number of T++ and T+ lesions per body area was noted and their minimum density (MD; minimum number of lesions per animal) for a certain period was recorded as low (1 to 5 lesions), medium (6 to 10) or high (>10).

Tattoo lesions were sampled in the dead juvenile SIC and fixed in 10% formaldehyde. However, the samples were too decomposed to allow the examination of histologic alterations. No cetacean poxviruses had been isolated or characterised at the time of writing, and specific antisera or primers were not available to detect virus antigens or amplify fragments of its genome. Thus, the etiologic agent could not be identified.

Statistics. For statistical analysis the data were grouped into 2 periods, 1994–1995 (first period) and 1996–1997 (second period), as the number of dolphins positive for T++ and T+ lesions varied between them. It was not possible to divide the data by year because the number of images for each dolphin per year was too limited, especially in the first period. Accordingly, a dolphin that had T++ or T+ lesions at some time during a period was considered positive for this period, though it may not have been positive for the whole period. We investigated 2 epidemiological patterns. In the first place, we examined for each period whether the prevalence of tattoos varied significantly with the age of the dolphins by grouping them into adult and immature (calves and juveniles) categories and using a 2-tailed Fisher's exact test. We considered all logical possibilities for the status of T+ lesions: (1) T+ are real tattoos and T+ dolphins are pooled with T++ individuals; (2) T+ are not tattoos and T+ dolphins are considered negative; (3) T+ are unknown lesions and T+ animals are excluded from the analysis. Lastly, we investigated whether the prevalence of tattoo positive dolphins varied significantly between the 2 periods using a McNemar test (Conover 1999). Statistical analysis was only possible if the T+ positive animals were considered as true T++ positives (Table 1). As the McNemar test could only be used for the dolphins that changed tattoo status between the 2 periods, and as this only occurred in adults (Table 1), statistical inference was limited to this age category. We used a 1-tailed test because a decrease in prevalence over time was expected in adults, as they may develop immunity against the virus and clear the disease (see Van Bressem & Van Waerebeek 1996).

To determine to what extent variation in the number and quality of the images could have biased the detection of differences in prevalence between immature and adult dolphins for each period, we used a univari-

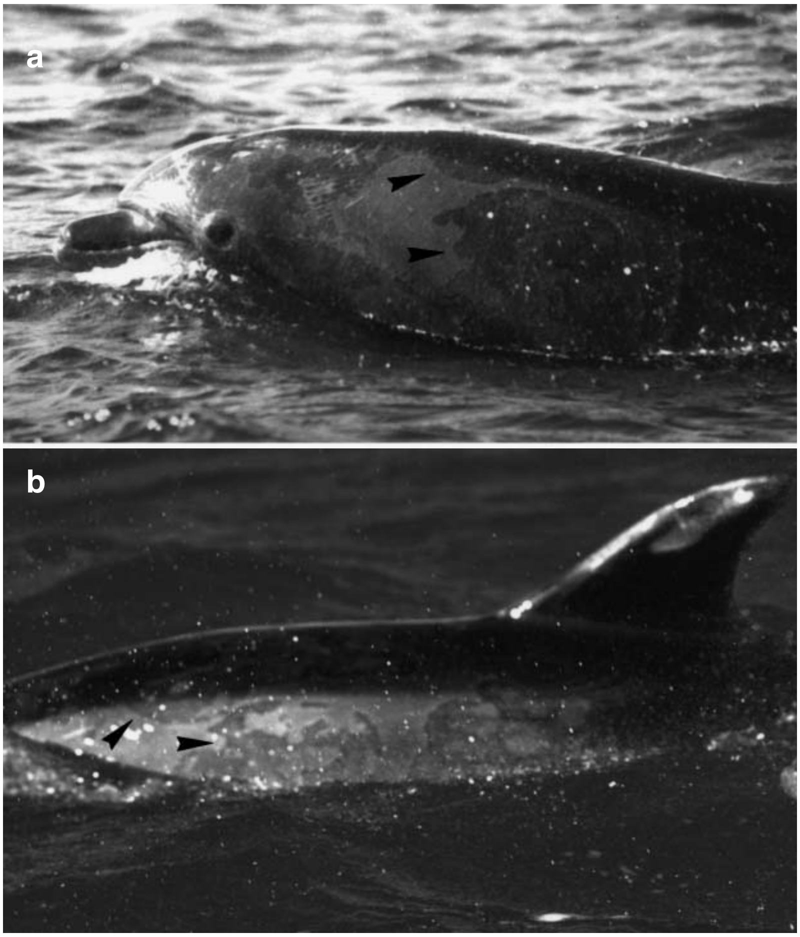

Fig. 2. *Tursiops truncatus.* (a) Some very large tattoo-like lesions observed on the back and flank of Dolphin TAL (arrowheads) in January 1997 which (b) converted into G-marks and split up (arrowheads) by December 1997

ate repeated-measures ANOVA, with 1 grouping factor (age group) and 1 'within' factor (quality category with 3 categories, i.e. good, average and poor). Likewise, to investigate if the differences in the number and image quality between the periods could have biased temporal changes of prevalence in adults, we used a univariate repeated-measures ANOVA with 2 'within' factors (quality category and period) (see Wilkinson & Coward 1996).

RESULTS

Images and detection of tattoos and tattoo-like lesions

A mean (±SD) of 16.7 ± 6.4 images (range 6 to 30) per animal was examined in 1994–1997. The number of GI, AI and PI for each dolphin and period is given in Table 1. Dolphins positive for T++ lesions were only seen in 19 GI. Dolphins with T+ lesions were observed in 39 GI, 23 AI and 6 PI. A mean of 2.5 ± 1.6 GI (range 0 to 6) images was available for each dolphin in 1994–1995 and 5.87 ± 3.4 GI (range 1 to 11) in 1996–1997.

Age variation in prevalence of tattoo disease

All juveniles, including the oldest calf (EAG) that turned juvenile during the course of the study, had true tattoos in both periods. More variability was observed in adults (Table 1). Considering the 3 possibilities regarding the status of T+ lesions, prevalence of tattoo skin disease was significantly higher in immature than in adult dolphins during both periods, except in the first one when T+ lesions were considered as true tattoos (Table 2). In 1994–1995, there were no biases associated with the sampling effort or due to the differences in image quality. Indeed, the global number of pictures was similar between the 2 age groups ($F_{1,30}$ = 0.04, p > 0.8), and the interaction between age group and image quality was not significant ($F_{2,60}$ = 0.15, p > 0.8). In 1996–1997, the global number of pictures did not vary significantly between the 2 age groups ($F_{1,29}$ = 2.11, p > 0.15) but there was a significant interaction between age group and image quality ($F_{2,58}$ = 8.74, p < 0.001). A post-hoc comparison revealed that the differences occurred only in the number of GI ($F_{1,29}$ = 12.12, p = 0.002), i.e. there were more GI in immature dolphins than in adults (Table 1).

Temporal variation in prevalence of tattoo disease

Minimum prevalence of T++ lesions in the dolphins from the Sado estuary was 21.9 % (N = 32) in 1994–1995 and 15.6 % (N = 32) in 1996–1997. When T+ lesions were considered as true tattoos, this prevalence reached 46.9 % in 1994–1995 and 31.3 % in 1996–1997. In the 23 adults examined during both periods, the prevalence of T++ and T+ lesions was significantly higher in 1994–1995 (39.1 %) than in 1996–1997 (17.4 %) (McNemar test, χ^2 = 3.2, df = 1, p = 0.03). The

Table 1. *Tursiops truncatus.* Data on the survey for tattoo skin disease in dolphins from the Sado estuary, Portugal. G = good, A = average, P = poor, INA = images not available (dolphin unborn, not photographed, or dead), NS = necropsy slides, N = absence of tattoos (T++) and tattoo-like (T+) lesions. Dolphin codes are for individual identification

Dolphin	Number of G, A and P images						Presence of T++ and T+ lesions	
	1994–1995			1996–1997			1994–1995	1996–1997
	G	A	P	G	A	P		
Immature								
BOL	0	1	0	10	3	1	N	N
ESP[a]	INA	INA	INA	11	2	2	INA	N
ZOE	0	6	1	5	6	5	N	N
ECL	INA	INA	INA	4	5	4	INA	T+
ESC	INA	INA	INA	9	4	2	INA	T+
EAG	2	4	1	8	4	2	T++	T++
APA	3	0	0	11	5	1	T++	T++
ORL	3	3	0	INA	INA	INA	T++	INA
SIC	4	2	0	NS	NS	NS	T++	T++
SUP	5	0	1	14	5	2	T++	T++
Adult								
AGU	3	3	0	5	4	4	N	N
BUM	1	2	1	4	4	4	N	N
FUG	1	2	0	1	2	1	N	N
JAN	6	0	0	4	7	3	N	N
LIS	3	3	0	INA	INA	INA	N	INA
QUA	0	1	0	3	4	2	N	N
COV	2	0	2	2	2	3	N	N
CUR	0	1	1	2	5	5	N	N
ELE[a]	3	1	0	3	10	0	N	N
FAC	4	1	0	4	3	3	N	N
FAR	2	2	0	2	5	2	N	N
MIL	0	3	0	1	5	2	N	N
RED	3	1	0	8	7	4	N	N
TRU	2	3	0	3	7	3	N	N
TUB	1	1	0	3	5	6	N	N
CAR	3	2	1	INA	INA	INA	T+	INA
GOR	3	1	0	7	4	2	T+	N
THO	4	2	0	4	5	2	T+	N
TOQ[a]	3	2	0	7	2	1	T+	N
TIP[a]	5	2	0	7	5	8	T+	N
HUB	3	3	1	11	3	1	T+	T+
MUR	1	4	0	6	9	4	T+	T+
UMM[a]	2	2	0	7	7	3	T+	T+
LUA	4	3	1	9	6	1	T++	N
TAL	4	2	0	7	8	5	T++	T+

[a]Year when a total of 10 (range 1 to 3) pictures from these dolphins was taken is uncertain (1995 or 1996)

Table 2. *Tursiops truncatus.* Significance of the age variation in prevalence of tattoo disease in dolphins from the Sado estuary, for each period, and considering the 3 logical possibilities regarding the status of tattoo-like (T+) lesions (2-tailed Fisher's exact test, df = 1)

Period	Age class	Status of T+ lesions					
		T+; true tattoos Prevalence (%)	p	T+; not tattoos Prevalence (%)	p	T+; unknown lesions Prevalence (%)	p
1994–1995	Immature	71.4 (N = 7)	0.2095	71.4 (N = 7)	0.0019	71.4 (N = 7)	0.0086
	Adult	40 (N = 25)		8 (N =25)		11.8 (N = 17)	
1996–1997	Immature	66.7 (N = 9)	0.0126	44.4 (N = 9)	0.007	57.1 (N = 7)	0.0047
	Adult	17.4 (N =23)		0 (N = 23)		0 (N = 19)	

Fig. 3. *Tursiops truncatus.* Superinfected tattoo-like lesions on the back, flank and head of Calf ESC in April 1997

overall number of pictures of adults varied significantly between periods ($F_{1,22}$ = 148.5, p < 0.0001), being higher in the second one (Table 1). The interaction between age quality and period was not significant ($F_{2,44}$ = 3.17, p > 0.05). The higher number of pictures in 1996–1997 may have favoured the detection of T++ and T+ lesions in this period and, thus, may further confirm a true decrease in prevalence among adults during this period.

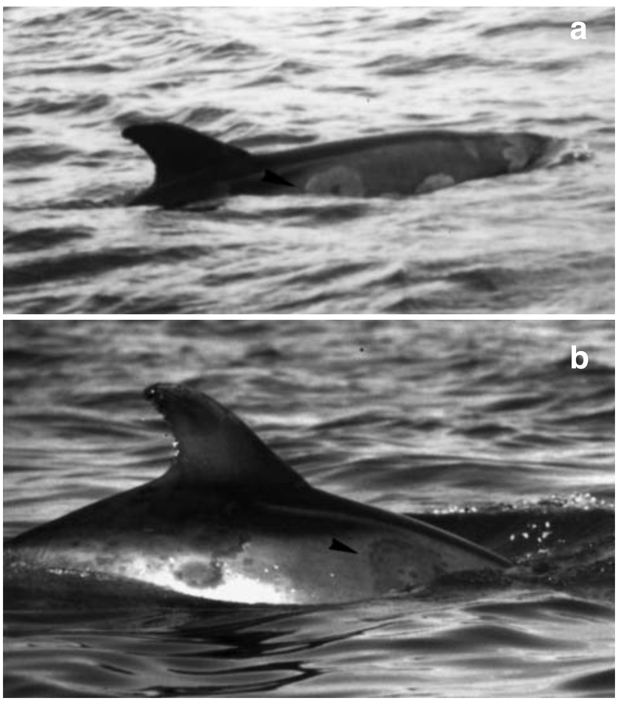

Fig. 4. *Tursiops truncatus.* Recurrence of tattoo disease: (a) G-marks on the flanks of Dolphin HUB in January 1995 (arrowhead); (b) tattoo-like lesions at the periphery of, and inside, one of these marks (arrowhead) in January 1997

Characteristics of the disease

T++ and T+ lesions were observed on the flanks, back, tailstock, head and dorsal fin of the dolphins (Table 3). Obviously, other body parts poorly or not at all photographed during this study could also have been affected. In dead juvenile SIC, 3 medium tattoos located on the head measured 61 × 61 mm, 60 × 40 mm, and 50 × 39 mm. In the other dolphins the relative size of the lesions varied between small and very large (Figs. 1a, 2a & 3, Table 3). The very large lesions were only observed in 2 adult dolphins that suffered the disease for at least 3 yr and in a calf (Figs. 2a & 3, Table 3). The MD of T++ and T+ lesions varied from low to high (Table 3). Individual T++ and T+ lesions may persist for months and even years (Table 3). While some heal, others may persist for longer periods in an affected dolphin. The MPT of the disease ranged from 3 to 45.5 mo (Table 3). A seemingly healing process was observed in 12 dolphins: the lesions converted into light grey marks (G-marks) that may or may not have a darker outline and a darker centre (fading pattern; Fig. 1). In one of these dolphins (TAL), the G-marks seemed to have split up (Fig. 2, Table 3) and, in at least one other animal (HUB), small and medium T+ lesions were again observed at the periphery or in the centre of G-marks after an apparent period of healing, suggesting recurrence of the disease (Fig. 4, Table 3). The T+ lesions observed in March 1994 on the back and flank of Dolphin GOR seemed to have completely healed by June 1996. In Calf ESC, the T+ lesions seemed to have been superinfected by an unknown agent (Fig. 3) that gave them an unusual aspect: light grey velvety marks surrounded by a dark grey border. These lesions grew very large and fused over a period of 3 mo and then converted into G-marks.

Table 3. *Tursiops truncatus*. Characteristics of tattoo disease in dolphins from the Sado estuary. MD = minimal density, MPT = minimal persistence time, F(s) = flank(s), D = dorsal fin, B = back, H = head, Ts = tailstock, FP-G = fading pattern (G-marks), NOB = not observed, TRE = total regression of tattoo-like lesions

Dolphin	MD of T++ and T+ lesions	Topography of lesions	Size of lesions	MPT of individual T++ and T+ lesions (mo)	General pattern of regression of lesions	MPT of tattoo disease (mo)
Positive for tattoo lesions						
Immature						
APA	High	B, Fs	Small to large	25	FP-G	25
EAG	High	B, H, F	Small and medium	14	FP-G	35
SIC[a]	High	B, Fs, Ts, H	Small to large	17–18	NOB	23.5
ORL[a]	High	Fs, B, H	Small to large	8	FP-G	17–18
SUP	High	Fs, B, H	Small to large	37	FP-G and possible recurrence	45.5
Adult						
LUA	High	Fs, B	Small to large	8	FP-G	8
TAL	High	Fs, H, B,	Small to very large	35	FP-G and splitting up	37
Positive for tattoo-like lesions						
Immature						
ECL	Low	B	Small	3	NOB	3
ESC	High	H, Fs, B	Medium to very large	3	FP-G	3
Adult						
CAR[a]	Low	B	Small and medium	11.5	NOB	11.5
GOR	Low	F, B	Medium	NOB	TRE	NOB
HUB	High	Fs, B, D	Small to very large	NOB	FP-G and recurrence	36[b]
MUR	Medium	F, B, H	Small to large	7	NOB	26–38
THO	High	Fs, B	Small to medium	NOB	FP-G	18–19
TIP	Medium	F, B, H	Small to large	NOB	FP-G	11–12
TOQ	Low	H	Large	NOB	FP-G	NOB
UMM	Low	F, B	Medium to large	12	FP-G	12

[a]These dolphins died in 1995–1996
[b]During that time the lesions regressed then recurred

Natural history of tattoo disease

In the 2 youngest T+ positive immatures, the lesions were first detected when they were 9 (ESC) and 14 (ECL) mo old. The earliest pictures of these animals examined during this study had been taken when they were 1 (ESC) and 2 to 3 (ECL) mo old. T++ or T+ lesions were not detected in the known mothers (BUM, ELE, TRU) of 3 positive immature dolphins (ECL, ESC, EAG). Three (CAR, ORL, SIC) of the 5 dolphins that died during the study period were positive for T++ and T+ lesions (Table 1).

DISCUSSION

This study describes, for the first time, the epidemiology of tattoo skin disease in live, free-ranging bottlenose dolphins monitored by photography over a 4 yr period, as described by Thompson & Hammond (1992) and Wilson et al. (1997). It also provides unique data on the patterns of regression of tattoo lesions as well as on the minimal persistence time of individual lesions and the disease. The only other study on the epidemiology of tattoo disease was carried out in small cetaceans that had died in fisheries off Peru in 1993–1994 (Van Bressem & Van Waerebeek 1996). Because of the design of the present study, the whole body of the dolphins could not been examined, and the status of T+ lesions could not be unequivocally determined. Thus, only the minimum prevalence levels of the disease could be determined for each period and age category, and different possibilities regarding the status of T+ lesions had to be considered for the statistical analysis. Though investigations on the aetiology of tattoo disease in the dolphins from the Sado estuary could not be carried out, it is likely that it was caused by a poxvirus. Indeed, poxviruses were the only infectious agents conspicuously observed by electron microscopy in tattoos from several species of odontocetes, including the bottlenose dolphin (for a review see Van Bressem et al. 1999), and were demonstrated in all tattoo samples from 11 long-beaked common dolphins, 2 dusky dolphins and 8 Burmeister's porpoises caught off central Peru from 1991 to 1995 (Van Bressem et al. unpubl. data).

Minimum prevalence of T++ lesions in all the resident bottlenose dolphins from the Sado estuary was 21.9% in 1994–1995 and 15.6% in 1996–1997. When T+ lesions were pooled, this prevalence reached 46.9% in 1994–1995 and 31.3% in 1996–1997. Prevalence of tattoo disease varied between 34.7 and 62.3% in the dolphins and porpoises from Peru (Van Bressem & Van Waerebeek 1996). Altogether these data indicate that when present in a population of small cetaceans, tattoo disease affects a high proportion of individuals. During the present study, T++ and T+ lesions were observed in calves (the youngest calf with T+ lesions was 9 mo old), juveniles and adults. However, prevalence of tattoo lesions tended to be significantly higher in immatures than in adults. In 1994–1995, this age variation was statistically significant (except when T+ lesions were considered to be true T++), while in 1996–1997 it was statistically significant regardless of the status of T+ lesions (Table 2). Though in 1996–1997 the significantly higher number of GI in immature dolphins may have favoured the detection of T++ lesions in this age class, the influence of this bias was negligible. Indeed, none of the immature dolphins examined in the first period changed category in the second period, and only T+ lesions were observed in the 2 young calves born in the second period (Table 1). In the Peruvian Delphinidae, prevalence of tattoo lesions was also higher in sexually immature than in mature individuals (Van Bressem & Van Waerebeek 1996). The presence of tattoo disease in all juvenile bottlenose dolphins is probably related to the loss of their passive immunity against the virus, together with a higher risk of close contact with infected pod members consequent to their straying behaviour, as described by Van Bressem & Van Waerebeek (1996). The lower prevalence of tattoo disease in adult dolphins is probably due to the development of an immune response against the virus. The eventual clearance of the disease in adult dolphins from the Sado estuary likely caused the significant variation in prevalence between the 2 periods (assuming in this case that T+ lesions are true tattoos).

In most affected dolphins from the Sado estuary, T++ and T+ lesions converted into light grey marks when healing. Individual T++ and T+ lesions may persist for months or even years in these dolphins, and recur on the same animal. Similar observations have been reported in captive bottlenose dolphins (Sweeney & Ridgway 1975, Geraci et al. 1979, Smith et al. 1983). In 2 captive bottlenose dolphins, recurrence of the disease was associated with stressful conditions that may have affected their immune response (Geraci et al. 1979, Fair & Becker 2000). The presence of very large T+ lesions in 2 adult dolphins (HUB and TAL) that suffered from the disease for at least 3 yr, in one case with an apparent period of regression followed by recurrence of the lesions, may indicate immunological deficiencies. Environmental contaminants, such as the polyhalogenated aromatic hydrocarbons (PHAHs), are immunotoxic and may contribute to the severity of diseases in marine mammals (Aguilar & Borrell 1994, Ross et al. 1996). Some of these, the organochlorines, have also been suggested to be associated with a higher prevalence of tattoo disease in inshore than in offshore and offshore-neritic adult Delphinidae (Van Bressem et al. 2003). The Sado estuary suffers from eutrophication and pollution from mining, industrial and agricultural activities, as well as from domestic sewage (Ferreira et al. 1989, Bruxelas et al. 1992, Harzen 1995). Further studies should examine the concentration of PHAHs in the tissues of bottlenose dolphins from the Sado estuary and evaluate their influence on the prevalence and evolution of tattoo disease.

In the dolphins from the Sado estuary, tattoo disease was first observed in a picture of Dolphin HUB taken in June 1992 (see Harzen & Brunnick 1997, p. 62), and could still be seen in at least 3 dolphins (TAL, SUP and HUB) in May 2003 (R. Gaspar unpubl. data). The high prevalence of the disease, its long persistence in some individuals, as well as its higher prevalence in immature dolphins strongly suggest that it is endemic in these Delphinidae (see Nathanson 1990). The community of resident bottlenose dolphins from the Sado Estuary is very small and declining (R. Gaspar et al. unpubl. data). Recruitment, namely juvenile survival, has been particularly low during the 1980s and early 1990s (Gaspar & Hammond 2001). The contribution of tattoo disease to this low recruitment is unknown and needs to be investigated. Indeed, all juveniles (including EAG) seemed to be severely affected by the disease, as indicated by a high MD of small to large tattoos on at least 3 body areas, and 2 of them died of unknown causes during the study. Though tattoo disease does generally not seem to affect the long-term health of odontocetes, a captive cetacean died after developing many tattoos over its body (Sweeney & Ridgway 1975). Tattoo disease was also suspected to cause mortalities among neonates and calves without protective immunity in the dusky dolphin and Burmeister's porpoise from Peru (Van Bressem et al. 1999).

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LITERATURE CITED

Aguilar A, Borrell A (1994) Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. Sci Total Environ 154:237–247
Bruxelas A, Cabeçadas L, Rosado C (1992) Recursos Marinhos e Poluição no estuário do Sado. In: Estudos de Biologia e Conservação da Natureza, Vol 6. Instituto da Conservação da Natureza, Lisbon
Conover WJ (1999) Practical nonparametric statistics, 3rd edn. John Wiley & Sons, New York
dos Santos ME, Lacerda M (1987) Preliminary observations of the bottlenose dolphin (*Tursiops truncatus*) in the Sado estuary (Portugal). Aquat Mamm 13:65–80
Fair PA, Becker PR (2000) Review of stress in marine mammals. J Aquat Ecosyst Stress Recov 7:335–354
Ferreira AM, Castro OG, Vale C (1989) Factors controlling PCB and DDT variations in the upper Sado estuary. Int Symp Integrated Approaches to Water Pollution Problems. 19–23 June 1989, Lisbon. Vol 2, p 83–92
Gaspar R (1994) Estudo dos movimentos, da sociabilidade e dos padrões de frequentação dos roazes *Tursiops truncatus* na região do estuário do Sado, utilizando fotoidentificação. BSc thesis, University of Lisbon
Gaspar R (1995) Observations on the occurrence of skin lesions in the bottlenose dolphins resident group from Sado Estuary region, followed through photographic data. In: Evans PGH, Nice H (eds) Proc 9th Annu Conf Eur Cetacean Soc, Lugano, Switzerland, February 1995. European Society for Cetacean Research, Kiel, p 224–226
Gaspar R (2000) Roazes do Sado — uma população ameaçada. In: Histórias da conservação da natureza. Instituto da Conservação da Natureza, Lisbon, p 19–22
Gaspar R, Hammond P (2001) Survival estimates for a very small bottlenose dolphin population. 14th Biennial Conference on the Biology of Marine Mammals, Vancouver, 28 December 2001 (Abstract)
Gaspar R, Van Bressem MF (1998) Poxvirus infection in bottlenose dolphins from the Sado Estuary, Portugal. The World Marine Mammal Science Conference, Monaco, January 1998 (Abstract)
Geraci JR, Hicks BD, St Aubin DJ (1979) Dolphin pox: a skin disease of cetaceans. Can J Comp Med 43:399–404
Harzen S (1995) Behaviour and social ecology of the bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821), in the Sado estuary, Portugal. PhD thesis, University of Bielefeld
Harzen S (1998) Habitat use by the bottlenose dolphin (*Tursiops truncatus*) in the Sado estuary, Portugal. Aquat Mamm 24(3):117–128
Harzen S, Brunnick B (1997) Skin disorders in bottlenose dolphins (*Tursiops truncatus*), resident in the Sado estuary, Portugal. Aquat Mamm 23:59–68
Nathanson N (1990) Epidemiology. In: Fields BN, Knipe DM, Chanock RM, Hirsch MS, Monath TP, Roizman B (eds) Virology, 2nd edn. Raven Press, New York, p 267–291
Ross PS, De Swart RL, Van Loveren H, Osterhaus ADME, Vos JG (1996) The immunotoxicity of environmental contaminants to marine wildlife: a review. Annu Rev Fish Dis 6: 151–165
Scott MD, Wells RS, Irvine AB (1990) A long term study of bottlenose dolphins on the west coast of Florida. In: Leatherwood S, Reeves RR (eds) The bottlenose dolphin. Academic Press, San Diego, p 235–244
Smith AW, Skilling DE, Ridgway SH, Fenner CA (1983) Regression of cetacean tattoo lesions concurrent with conversion of precipitin antibody against a poxvirus. J Am Vet Med Assoc 183:1219–1222
Smolker RA, Richards AF, Connor RC, Pepper JW (1992) Sex differences in patterns of association among Indian Ocean bottlenose dolphins. Behaviour 123(1–2):38–69
Sweeney JC, Ridgway SH (1975) Common diseases of small cetaceans. J Am Vet Med Assoc 167:533–540
Thompson PM, Hammond PS (1992) The use of photography to monitor dermal disease in wild bottlenose dolphins *Tursiops truncatus.* Ambio 21(2):135–137
Thrusfield M (1986) Veterinary epidemiology. Butterworths, London
Van Bressem MF, Gaspar R (1999) Epidemiology of tattoo skin disease in bottlenose dolphins from the Sado Estuary region, Portugal. 13th Biennial Conference on the Biology of Marine Mammals, Maui, Hawaii, November 1999 (Abstract)
Van Bressem MF, Van Waerebeek K (1996) Epidemiology of poxvirus in small cetaceans from the Eastern South Pacific. Mar Mamm Sci 12:371–382
Van Bressem MF, Van Waerebeek K, Reyes JC, Dekegel D, Pastoret PP (1993) Evidence of poxvirus in dusky dolphin (*Lagenorhynchus obscurus*) and Burmeister's porpoise (*Phocoena spinipinnis*) from coastal Peru. J Wildl Dis 29: 109–113
Van Bressem MF, Van Waerebeek K, Bennett M (1998) Cetacean poxviruses are antigenically related to cowpox virus. The World Marine Mammal Science Conference, Monaco, January 1998 (Abstract)
Van Bressem MF, Van Waerebeek K, Raga JA (1999) A review of virus infections of cetaceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. Dis Aquat Org 38:53–65
Van Bressem MF, Van Waerebeek K, Raga JA, Gaspar R, Di Beneditto AP, Ramos R, Siebert U (2003) Tattoo disease of odontocetes as a potential indicator of a degrading or stressful environment: a preliminary report. Document SC/55/E1, International Whaling Commission, Berlin
Wells RS, Scott MD (1990) Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. Rep Int Whal Comm (Spec Iss 12) 407–415
Wilkinson L, Coward M (1996) Analysis of variance. In: Statistics. SYSTAT 6.0 for Windows. SPSS, Chicago, p 119–223
Wilson B, Thompson PM, Hammond PS (1997) Skin lesions and physical deformities in bottlenose dolphins in the Moray Firth: population prevalence and age-sex differences. Ambio 26:243–247
Wilson B, Arnold H, Berazi G, Fortuna CM and 12 others (1999a) Epidermal lesions in bottlenose dolphins: impacts of natural and anthropogenic factors. Proc R Soc Lond B 266:1077–1083
Wilson B, Hammond PS, Thompson PM (1999b) Estimating size and assessing trends in a coastal bottlenose dolphin population. Ecol Appl 9:288–300
Würsig B, Jefferson RA (1990) Methods of photo-identification for small cetaceans. Rep Int Whal Comm (Spec Iss 12) 42–43

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